Editorial Commentary: Is the In Vitro Interferon- Release Assay an Adequate Replacement for the Tuberculin Skin Test?

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Is the In Vitro Interferon-γ Release Assay an Adequate Replacement for the Tuberculin Skin Test?

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(See the article by Bellete et al. on pages 1449–56)

Tuberculosis takes an enormous toll on humankind. The impact of *Mycobacterium tuberculosis* has not been weakened despite the availability of effective antituberculosis agents. Industrialized countries recorded a net decline in the incidence of tuberculosis during the 20th century, but, unfortunately, developing countries continue to witness the spread of this disease at a frightening and increasing pace. On the verge of the 21st century, >8 million people globally were newly infected with *M. tuberculosis,* 95% of them in developing countries, and 2 million deaths resulted from uncontained infections per year. Thus, tuberculosis remains a leading killer [1].

The key to the success of *M. tuberculosis* is its unique ability to adapt to a wide range of conditions, both inside and outside the human host [2]. Infection with *M. tuberculosis* is most often acquired by inhalation of small-particle droplets that contain the bacterium. Once the pathogen reaches the alveoli, it undergoes phagocytosis by alveolar macrophages. Some bacilli may, however, resist destruction and replicate within phagocytic cells. In response, macrophages produce cytokines, including TNF-α, IL-12, and multiple chemokines. The latter act as chemoattractants to recruit neutrophils, monocytes, and lymphocytes. TNF-α and IL-12 facilitate IFN-γ production by natural killer cells and, subsequently, by T lymphocytes. Although these innate pulmonary defense mechanisms limit the initial replication and spread of invading mycobacteria, the extent of success varies with the virulence and number of infecting microorganisms. At this stage, all mycobacteria rarely are eliminated. Some infected macrophages and dendritic cells migrate to regional lymph nodes, where the antigen-specific host response is initiated. It appears that γδ T lymphocytes are the predominant T cell population in this phase of the immune response. They produce a broad spectrum of cytokines, including IFN-γ, IL-2, IL-4, IL-5, and IL-10, and can lyse infected macrophages. A second wave of T cells (αβ T lymphocytes) is attracted to and activated by the cytokines produced by γδ T lymphocytes. These complementary T cell populations produce cytokines and chemoattractants that recruit uncommitted lymphocytes, and the lymphocytes contribute indirectly to mycobacterial elimination by macrophage activation. Clones of *M. tuberculosis*–reactive $CD4^+$ T cells are expanded, some of which differentiate to memory T lymphocytes. These cells mediate delayed-type hypersensitivity and, together with *M. tuberculosis*–specific $CD8^+$ T cells and CD1b-restricted T cells that recognize mycobacterial lipids [3], initiate the secondary immune response displayed in lymphoid tissue, lung parenchyma, and metastatic foci. IFN-γ is the key effector cytokine in control of mycobacterial infection via macrophage activation [4].

The efficacy of the secondary immune response likely determines whether active infection is terminated or progressive disease evolves. The most common outcome of primary infection with *M. tuberculosis,* however, is clinical latency, which represents a balance between the host's cell-mediated immune response and the ability of mycobacteria to slow down replication and wait patiently (dormancy). Currently, 2 billion people, 33% of the world's population, are estimated to be infected with *M. tuberculosis,* which provides an immense reservoir for the pathogen, and 16 million of these subjects exhibit active tuberculosis.

Latently infected persons represent a potential danger. Impairment of cellular immunity predisposes the host to reactivation of latent infection and manifestation of disease. It is at this point that the mycobacterial strategy of "winning by waiting" pays off, because hosts with active

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infection are capable of transmitting *M. tuberculosis* [2]. One dimension of the danger attending latent infections has been revealed by the ongoing HIV epidemic. The dangerous liaison between HIV and *M. tuberculosis* resulted in the resurgence of tuberculosis in developed countries in the late 1980s and early 1990s [5] and in the unprecedented acceleration of the spread of the disease in developing countries. Other factors impairing cellular immunity include malnutrition, use of immunosuppressive drugs, and senescence.

The only way to halt the spread of tuberculosis is to interrupt the chain of infection. This requires that all infected persons be identified and receive effective antimicrobial treatment. A relatively simple diagnostic armamentarium suffices for recognition of actively infected and highly contagious persons, even in developing countries. Acid-fast staining and microscopic examination are sufficient to detect the pathogen in the sputum of adults. Culturing allows species identification and determination of susceptibility to antimicrobial agents to be accomplished. On the other hand, diagnosis of pulmonary tuberculosis is hampered if *M. tuberculosis* organisms are not excreted in sputum in numbers sufficient to be readily detected by smear microscopy ($\sim 10^4$ cells/mL) or if sputum cannot be obtained for microbiological investigation (e.g., from children). Thus, for these patients, diagnosis demands additional diagnostic tools, as does the identification of latently infected persons.

The tuberculin skin test (TST) represents such an additional tool [6]. It assesses the degree of cellular immune response to PPD of *M. tuberculosis.* In sensitized persons, intradermally inoculated PPD elicits induration at the injection site within 48–72 h. The size of the induration depends on the number of infiltrating and accumulating cells during this period of time. The majority of these cells are memory $CD4^+$ T helper lymphocytes. In sensitized persons, they constantly migrate in the bloodstream, snooping around for mycobacterial antigens. However, the accuracy of the TST depends on several factors. Most critical are errors in placement and interpretation, which can lead to either overestimation or underestimation of sensitization. Contact with environmental mycobacteria other than *M. tuberculosis,* as well as previous vaccination with BCG, may cause false-positive results. Inherited or acquired immunodeficiency, iatrogenic immunosuppression, concomitant infections, and a high mycobacterial load blunt intradermal responses. Given the several drawbacks associated with the TST, more objective and reliable tools for indirect detection of infection with *M. tuberculosis* are highly desirable.

Much attention has been devoted recently to the IFN-γ release assay (IGRA), an in vitro assay marketed in Australia. The assay is based on quantification of IFN-γ released by peripheral blood cells after stimulation with PPD from *M. tuberculosis* and control antigens. Recently, Mazurek et al. [7] reported the results of a multicenter study that included >1200 adults in the United States and compared the IGRA with the TST for detection of latent *M. tuberculosis* infection. The authors concluded that the IGRA and TST were comparable in their ability to detect latent tuberculosis. Unfortunately, this conclusion was not supported by the results presented. Overall agreement between the IGRA and TST was 83%, and agreement of the tests among persons at risk for latent infection ranged from 89.9% to 92.7% for those with negative results of the TST. However, agreement was <65% for those with positive results of the TST, regardless of whether the subjects had been vaccinated with BCG. Notably, efforts to minimize the subjectivity associated with reading TST results were, apparently, not undertaken, because some participating centers practiced digit preference (i.e., rounding measurements of TST induration to the nearest multiple of 5 mm). The authors also favored the IGRA because of its logistical advantage over the TST in requiring a single patient visit. However, this argument does not hold true, because patients need to be told about the results of the IGRA, and those with latent tuberculosis need to have antimicrobial treatment prescribed and to receive detailed information about it. Therefore, a second visit would be required, at least for those patients with latent tuberculosis. With the TST, the reading of the results and treatment prescription are done efficiently at the same visit.

In this issue of *Clinical Infectious Diseases,* Bellete et al. [8] present an important investigation of the IGRA's performance among 2 populations with different backgrounds with regard to the prevalence of tuberculosis. One population originated from Baltimore and had varying levels of risk of exposure to *M. tuberculosis,* and the other consisted of subjects from Addis Ababa, Ethiopia, where tuberculosis is highly endemic. Volunteers from Baltimore had participated in the reported US multicenter trial [7]. Two essential issues were addressed. First, for the IFN-γ response, breakpoints other than those recommended by the manufacturer were examined. TST induration diameters showed a bimodal distribution, but no such modality was found for the IFN-γ responses. Thus, although the distribution of TST induration values allowed a clear distinction to be made between positive and negative reactions, it was not possible to designate a cutoff point for the IGRA. Second, the reproducibility of the IGRA results was assessed. Baltimore subjects with discordant TST and IGRA results were asked to participate in repeat testing. Changes in TST results were compatible with booster phenomena. By contrast, changes in IGRA results were random. Overall, the IGRA performed with poorer sensitivity and specificity than did the TST.

The IGRA is an attractive diagnostic tool. However, studies assessing its performance have not provided convincing results. Before we can become excited about the IGRA, various issues need to be

resolved. The sensitivity and specificity of the assay may be increased by use of more apt and specific stimulatory antigens [6]. Reproducibility may be improved and interpretation may be facilitated if host and environmental factors that influence the IGRA, including age, immune status, concomitant infections, and medications, are clearly defined. IFN-γ is a key component of the host response to *M. tuberculosis.* Nevertheless, immune reactions to this bacterium are complex, and the spectrum of immune responses, which also are influenced by the genetic background [9], determines the outcome of *M. tuberculosis* infection. Indirect diagnosis of infection with *M. tuberculosis* by assessment of immune responses is, thus, prone to error. Importantly, low IFN-γ responses must not be considered to exclude the presence of infection. Risk-stratified interpretation of TST induration takes this into account. At this stage of development and with current knowledge, the IGRA can by no means replace the TST for detection of active or latent infection with *M. tuberculosis.*

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